PLANT NEMATOLOGY

LABORATORY MANUAL

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Prepared by the

Nematology Section

U.S.D.A.

1957

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Collecting Soil and Plant Samples

In collecting plant and soil samples for nematode examination, it should be kept in mind that the nematodes feed on roots of growing plants and can be most easily found where their food is abundant (Fig. 1).

A good way to proceed in sampling soil around the roots of growing plants is as follows:

1. Get a shovel, trowel, soil sampling tube or other digging implements. Also a supply of plastic (or paper) bags, and some labels.

2. Select the plant to sample. The best plants to sample are those which are not yet seriously damaged and still have plenty of living roots. Plants with mostly dead roots will not have many plant parasitic nematodes because there is little food for them.

3. Dig samples to include living roots and some of the adjacent soil. If the plants are small, dig up a whole root system. If the plant is larger, take only part of the root system. A sample of about 500 cc is sufficient.

4. Place the sample in the bag and label it.

5. Protect samples from drying or from excessive heat. Either may kill many of the nematodes.

6. Extract the nematodes from the samples as soon as possible, using the methods described below.

7. If the nematodes are in the plant tissue, such as roots, stems or bulbs, collect plants which show the symptoms and keep from drying until the nematodes are extracted.

Isolations of Nematodes from Soil Samples

Nematodes can be isolated from soil samples by: (1) the Cobb sieving and gravity method and (2) the Baermann funnel.

Sieving and Gravity Method for Soil

1. Put a soil sample of about 300 cc of soil in a bucket (Fig. 2A).
2. Add about 2 liters of water.
3. Mix the soil and water by stirring with a stick, then allow mixture to stand for 30 seconds.
4. Pour the water through a 20-mesh sieve into a second bucket (Fig. 2B).
5. Discard the debris on the 20-mesh sieve.

Note: A larger proportion of the nematodes can be obtained by repeating 2 to 5 once or twice.

6. Discard the material in the first bucket. This consists of sand and heavy soil particles.
7. Pour the water in the second bucket back through a 60-mesh sieve into the first bucket (Fig. 3C).
8. Wash the residue from the 60-mesh sieve into a beaker (Fig. 4E).
9. Pour the water in the first bucket through a 200-mesh sieve.
10. Wash the residue from the 200-mesh sieve into a beaker (Fig. 4E).
11. Pour the material in the beakers into shallow dishes for examination by the microscope. Cysts or adult females of the genus Heterodera will be found in the residue from the 60-mesh sieve. Cysts often float, so both the bottom of the dish and the surface of the water should be examined. Some of the larger nematodes will also be found in the residue from the 60-mesh sieve; the smaller nematodes will be found in the residue from the 200-mesh sieve. Or: Place it in the Baermann funnel as described below.

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Note: Ordinary soil sieves, obtainable from scientific supply companies, can be used for extracting nematodes by the sieving method. The "20-mesh" sieve has 20 openings to the inch and each opening 0.840 mm. wide. The "60-mesh" sieve has openings 0.250 mm. wide and the openings in the "200-mesh" sieve are 0.074 mm. wide.

The Baermann Funnel

A Baermann funnel is made by attaching a short piece of rubber tubing to a funnel and placing a pinchcock on the tubing. The funnel is then placed in an upright position and partly filled with water (Fig. 4F).

1. Place the sample from which the nematodes are to be extracted in a beaker.
2. Place a piece of cloth over the beaker and fasten it with a rubber band.
3. Invert the beaker in the funnel so that the cloth is under the surface of the water.
4. Leave the funnel undisturbed for 3 hours or longer.
5. Take a sample from the funnel by opening the pinchcock for a short time. The sample should not be more than 10 ml.
6. Place the sample in a shallow dish for examination.

Uses of the Baermann Funnel

The Baermann funnel can be used for extraction of nematodes from (1) soil samples, (2) sieve residues obtained by sieving soil as described above and (3) plant material.

For Soil Samples

1. Mix the soil samples thoroughly.
2. Put about 50 cc in a beaker.
3. Put a cloth cover over the beaker and fasten it with a rubber band.
4. Invert the beaker in the Baermann funnel.

For Sieve Residues

1. Place a cloth over the beaker containing the sieve residue and fasten it with a rubber band.

2. Invert the beaker in the Baermann funnel.

Plant Material

1. Cut the material into small pieces and place in a beaker.

2. Place a cloth over the beaker and fasten with a rubber band.

3. Invert the beaker in the funnel.

Notes

Extraction of nematodes by the Baermann funnel depends on active movements of the nematodes which work their way through the cloth. Dead nematodes will not be extracted by this method, so it cannot be used with preserved material. In addition to cloth, various kinds of paper have been used in the Baermann funnel. Paper towels work very well as do different kinds of thin porous paper.

Besides the method mentioned, there are numerous ways of supporting the cloth in the Baermann funnel. Wire rings, screen wire or clothes pins have been used.

Nematodes left too long in the Baermann funnel sometimes die from lack of oxygen.

Microscopes and Accessories

The nematologist needs two microscopes as follows:

1. Dissecting Microscope with stand and mirror for substage illumination, equipped with oculars and objectives to give magnifications of about 6 to 10X, 20 to 30X, and 50 to 75X. The lowest magnifications (about 6 to 30X) are used for examination of plant samples for symptoms of nematode attack, and for dissecting plant tissue to isolate the contained nematodes. These magnifications are also used in the various steps of preparing slides. Using the higher magnifications, the skilled worker can make tentative identifications of some kinds of nematodes.
2. Compound microscope equipped with a condensor, three objectives and oculars to give magnifications of about 100X, 450X and 950X. The usual combination is objectives of 16 mm., 4 mm., and about 2 mm. focal lengths, and oculars of about 10X. The lowest magnification is used for locating nematodes on the slide, and for observation of the general size and shape of the body. With magnifications of about 450X, some details of internal and external structure can be seen sufficiently well to permit identification to genus and sometimes to species. The highest magnification, obtained with an oil immersion objective, is used for study of minute details and is necessary for identification of most species.

A light which can be used for either incident or transmitted illumination is needed for the dissecting microscope, and a good microscope lamp is needed for the compound microscope.

An eyepiece micrometer or filar micrometer is a useful accessory for the compound microscope.

A camera lucida is needed if drawings of nematodes are to be made.

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Finding Nematodes in Plant Tissue

Nematodes in plant tissue may be (1) active nematodes or (2) inactive. Different methods are used in searching for each.

Active Nematodes

Active nematodes are able to move and are always slender. This kind of nematode can be found in plant tissue as follows:

1. Place a small piece of the plant tissue in a watch glass and cover with water (Fig. 5A).

2. Place the watch glass under the dissecting microscope, using a magnification of 10x to 30x.

3. With dissecting needles, tear the tissue apart (Fig. 6B).

4. This frees the nematodes from the plant tissue and they can then be found in the water. Look for them in the bottom of the dish.

5. If no nematodes are found, set the dish aside for an hour or more. Then look again.

A very efficient method of finding active nematodes in roots is the "Incubation Technique" described by Young in 1954 (Fig. 7).

1. Wash the roots thoroughly.

2. Place the wet roots in a fruit jar with a cover.

3. Set the jar aside for several days.

4. Spray a small amount of water on the roots and then pour it off into a beaker.

5. Examine this water under the dissecting microscope.

The Waring Blendor

When nematodes are numerous in plant tissue, they can be found as follows:

1. Place about 2 grams of washed plant material in a Waring Blendor and add about 100 ml. of water (Fig. 8A).

2. Run the Blendor for 20 seconds.

3. Pour the material through a 60-mesh sieve over a 200-mesh sieve (Fig. 8B).

4. Wash the material on the sieves with a gentle stream of tap water.

5. Discard the residue on the 60-mesh sieve and look for nematodes in the residue on the 200-mesh sieve.

6. Or: Place the residue from the 200-mesh sieve in the Baermann funnel.

Inactive Nematodes

Inactive nematodes are the species with "pear-shaped" or otherwise enlarged bodies, such as the root-knot nematodes. These will be partially or completely embedded in the plant tissue.

1. Place short pieces of the plant tissue in a watch glass under the dissecting microscope.

2. Using dissecting needles, pull the tissue apart to expose the nematodes.

3. Dissect the nematodes out of the plant tissue.

Note: Root-knot nematodes will be found mostly in roots with distinct knots, but are occasionally found in roots without distinct knots. The nematodes are in the knots, and their exact location is often marked by the presence of an egg mass. This is an irregular brown body about one millimeter in diameter on the surface of the root. It can easily be detached, revealing the nematode underneath.

Root-knot nematodes are more easily dissected out of roots which have been in 5% formalin for at least 24 hours than from fresh roots.

Mounting Nematodes on Temporary Microscope Slides

Microscope slides of nematodes which will be usable for several weeks or months can be made as follows:

1. Place a drop of 5% formaldehyde in the center of a clean microscope slide (Fig. 10A).

2. Locate a nematode in the Syracuse Watch Glass with the dissecting microscope, using a magnification of about 15 to 30X.

3. Gently lift the nematode with the point of the bamboo splinter upward to the water surface. Keep it in focus under the microscope (Fig. 9).

4. When the nematode is just under the surface of the water, lift rapidly to bring it through the surface film.

5. Place the point of the bamboo splinter in the drop of 5% formaldehyde on the slide and withdraw it carefully. This will usually leave the nematodes in the drop (Fig. 10A).

6. Place the slide under the dissecting microscope and make sure that all the nematodes are at the bottom of the drop.

7. Using forceps, carefully place a cover glass over the drop (Fig. 10B).

8. Still working under the microscope, use a small piece of filter paper, blotting paper or paper towel to absorb the excess liquid. Be careful that none of the nematodes are lost (Fig. 11A).

9. Seal the slide with either: (a) A mixture of one part of paraffin and one part of vaseline. This is kept hot and applied with a small brush. (b) ZUT, a special slide-sealing compound obtainable from Bennett's, 65 West First South Street, Salt Lake City 10, Utah. (c) Other materials including fingernail polish (Fig. 11B).

10. Label the slide (Fig. 11C).

Notes: A bamboo splinter suitable for picking up nematodes is made by splitting a piece of bamboo about 20 cm long to obtain a piece about 2 mm wide. One end of this is sharpened with a knife to make a long and very fine point. The point is best made by trimming with a razor blade under the dissecting microscope. The finer the point, the easier it is to use (Fig. 9).

Cover glasses should be the thinnest obtainable, usually the "No. 1" or "No. 0" thickness. If cover glasses are too thick, the nematodes cannot be examined with the oil immersion lens. Diameter of cover glasses should be 12 to 18 mm.

The slide sealing mixture is painted on, working under the dissecting microscope to be sure that the edges of the cover slip are sealed.

Other Methods of Making Microscope Slides of Nematodes

1. Temporary slides for immediate use under the lower powers of the compound microscope can be made by simply placing the nematodes in a drop of water and then placing a cover glass. If the nematodes are moving, heat the slide very gently with a match. Try to heat just enough to stop movement.

2. Slides can also be made by mounting nematodes which have been preserved in 5% formaldehyde in lacto-phenol solution. (Melted phenol, 3 parts; lactic acid, 1 part; glycerine, 2 parts; water, 1 part.) Such slides deteriorate in a few months so that certain internal structures of the nematode are difficult to see. Cuticle details remain good. This is an excellent mounting medium for cysts of *Heterodera* species and for perineal patterns of *Meloidogyne* species.

3. Permanent slides are made by mounting nematodes in glycerine. See below under "Fixing and Preserving Specimens".

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Fixing and Preserving Specimens

The standard material for preserving nematodes or plants and soil containing nematodes is 5% formaldehyde. A good procedure is as follows:

1. Prepare 10% formaldehyde by proper dilution with water of commercial formaldehyde solutions containing about 36% to 40% formaldehyde.

2. Place the material to be fixed in water at room temperature or below and add an equal volume of 10% formaldehyde solution heated to just below the boiling point. This relaxes and kills the nematodes without danger of ruining them by overheating.

3. Store in tightly closed containers until ready for examination. Or, transfer to pure glycerine as described below.

Note: Nematodes, or plant material containing nematodes will remain in good condition for years in 5% formaldehyde. Most other materials which have been tried have given inferior results in the long run. Formalin can also be used cold and this is sometimes an advantage if cuticular structures are to be examined. If sufficient material is at hand, it may be desirable to divide it and use both hot and cold formaldehyde.

4. Prepare solution of 1.5% glycerine in 10% ethyl alcohol.

5. Place the nematodes in this solution in Bureau of Plant Industry Watch Glasses or other small shallow containers. (Note B.P.I. Watch Glasses are Syracuse watch glasses 27 mm. in diameter.)

6. Place the watch glass in a tightly closed container of about 200 ml. capacity with a 16 mm. by 57 mm. screw cap vial filled with desiccated calcium carbonate. The cap of the vial should have a hole about 2 mm. in diameter. The purpose of this procedure is to permit very gradual evaporation of the water and alcohol, leaving the nematodes in pure glycerine after 3 to 6 weeks. If evaporation is too rapid, the nematodes will often collapse.

7. Transfer the watch glasses to a large desiccator containing calcium chloride until it is certain that all the water and alcohol have been evaporated. The nematodes can then be stored indefinitely in desiccated glycerine in tightly closed containers.

Making Permanent Slides

1. Keep a shallow dish of glycerin for use as the mounting medium and another dish containing short pieces of glass rod in the desiccator. The glass rods should have about the same diameter as nematodes, that is, should be of varying diameters in the range of 10 to 150 microns. These rods are obtained by cutting glass wool into lengths of several millimeters, or by drawing out threads from glass rods heated by a Bunsen burner.

2. Place a small drop of the mounting medium on a slide and transfer nematodes to it from the watch glasses.

3. Select three pieces of glass rod just a little smaller in diameter than the nematodes and place at the edges of the drops evenly spaced. These are to support the cover glass so that the nematodes are not flattened or distorted.

4. Lower a cover glass gently over the drop of mounting fluid.

5. Blot up excess glycerine from around the edges of the cover glass.

6. Seal the slide with ZUT.

Note: Nematodes can be mounted on ordinary microscope slides, but there is a considerable advantage in the use of the metal slides invented by Cobb (1917). These consist of a piece of aluminum bent to hold a square cover glass and 2 pieces of cardboard. Nematodes are mounted between the square cover glass and a round cover glass and so can be examined from either side. The cardboard pieces are used as labels. Metal slides can be stacked and so stored in boxes without the slots which are needed for glass slides.

Nematode Anatomy

All nematodes at some stage of their life are elongate worms, the body shape ranging from nearly cylindrical with rounded ends to fusiform. Most nematodes are this shape all through their lives, but adult females of certain important plant parasites have enlarged bodies which are pear-shaped, lemon-shaped, kidney-shaped, or otherwise enlarged (Fig. 12). The only appendages nematodes have are short setae usually near the anterior end. Soil and plant parasitic nematodes are rather small, average length being about a millimeter, with a minimum length of about 0.25 mm. and a maximum of about 10 mm. The great majority of the species are less than 2 mm. long.

In the typical nematodes (Fig. 14) the mouth is terminal at the anterior end. There is a mouth cavity which is reduced to a stylet in plant parasitic forms. Attached to the mouth cavity or stylet is the oesophagus which leads to the intestine. The intestine leads to the rectum and ends at the anus, which is situated on the ventral side. The body from the anus to the posterior terminus is the tail.

In the female, the vulva is also situated on the ventral side and is a transverse slit. This leads to the ovaries which are tubes lying in the body cavity. There may be either one or two ovaries. At the posterior end of the male body are a pair of structures known as spicules. These normally lie in the body, but can be pushed out through the anus. They are used in copulation. Also leading to the anus of the male is a single tube in which sperms are formed called the testis. Rearely, there are two testes.

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There is also a nervous system, the only visible part of which is the nerve ring around the oesophagus, and this is usually difficult to see.

The body is lined with muscle fibers arranged in four sectors separated by two lateral chords, a dorsal and a ventral chord. These are usually difficult to see.

Sensory organs include papillae surrounding the mouth cavity, near the excretory pore and near the posterior end. At the anterior end are also two chemical sense organs called amphids. In the Phasmidia these are terminal and very small so are seldom seen. In certain of the Aphasmidia, the external amphids are conspicuous circles, spirals and similar forms.

The phasmids are generally located in the tail. These are two glands connecting with lateral pores located in the middle of the lateral fields. Usually only the pores can be seen and often these are hard to locate. The canals leading to the surface can sometimes be seen in ventral or dorsal views.

The body of the nematode is covered with a transparent, colorless or slightly yellowish cuticle. This cuticle may be marked in various ways. Many nematodes have annules running at right angles to the body axis. These may be less than one micron wide or as much as 5 microns wide. Often they are interrupted laterally by the "lateral fields" which are more or less elevated ridges running lengthwise of the nematode. In many Tylenchida, the lateral fields are divided into three or more strips by lines.

In one genus of the Tylenchidae (Criconema) the coarse annules are bordered by well developed rows of spines or scales.

Nematode Systematics

Nematodes belong to the Phylum Nematoda according to some authors, and to the Phylum Aschelmintha according to others. It is generally agreed that they should be subdivided into 2 classes as suggested by Chitwood in 1933. These classes are (1) Phasmidia, and (2) Aphasmidia; that is, nematodes with phasmids and nematodes without phasmids.

The classes are divided into orders with names derived from that of the type genus and ending in "-ida" (example Rhabditida). Names of suborders end in "-ina" (Rhabditina); of superfamilies in "-oidea" (Rhabditoidea); of families in "idae" (Rhabditidae); and of subfamilies in "-inae" (Rhabditinae). The families are composed of genera and the genera of species. A few subspecies have been named.

About 5000 nematode species which are parasites of vertebrates are known. About an equal number of species of soil, marine, freshwater, insect parasites and plant parasites are known. This may be only a tenth or less of the existing species. The nematode fauna of the soil and plants of the greater part of the world is unknown.

In this manual we are concerned principally with the plant parasitic nematodes. Since the plant nematologist often encounters free-living nematodes in his work, we will also discuss the most common genera of these.

The plant parasitic forms now known belong mostly to a single order, the Tylenchida, of the class Phasmidia. A few species of 3 genera of the superfamily Dorylaimoidea of the Aphasmidia are also plant parasites. Soil nematodes are found in both Phasmidia and Aphasmidia.

The best book on taxonomy of the soil and plant parasitic nematodes is "Soil and Freshwater Nematodes", by T. Goodey. This was published in 1951 by Methuen & Co., Ltd., London and by John Wiley & Sons, Inc., New York. It contains description of the genera with which we are concerned and illustrations

of a representative species of each genus. The next best book is "A Manual of Agricultural Helminthology", by I. N. Filipjev and J. H. Schuurmans-Stekhoven, published by E. J. Brill, Leiden, Holland in 1941. This is obtainable from E. G. Stechert & Co., 31 East 10th Street, New York City.

With the keys in this manual and "Soil and Freshwater Nematodes", identification of the common soil and plant parasitic nematodes to genus is possible.

Nematode taxonomy is constantly growing and changing. New species of nematodes are constantly being found, and new genera are fairly common. In addition a certain amount of changing of species from one genus to another is constantly being done. Consequently, identification of nematodes to species is difficult and often nearly impossible unless the genus has been recently revised.

In using the keys, constant reference to the illustrations of this manual and to those of the reference books should be made. There is no substitute for illustrations.

Identification of Nematodes

The attached keys are used for nematode identification. The "Key to the most Common Nematodes of Agricultural Soils and Plants" will facilitate identification to family or subfamily.

As the first step a decision is made as to whether the nematode belongs to the Phasmidia or Aphasmidia. This is best determined by the form of the oesophagus. In the Phasmidia we find the oesophagi like those shown in figures 15 and 16.

Note that the "Rhabditoid" oesophagus has a distinct bulb at the base and that the bulb contains a valve of distinctive shape. This valve is easy to see (Fig. 15A).

The "Diplogasteroid" oesophagus is of the same general shape but the valve is lacking (Fig. 15B).

The other 3 types of oesophagi, "Tylenchoid", "Neotylenchoid" and "Aphelenchoid" are characterized by a stylet which is always present and easy to see. Stylets may be variations of any of the forms shown in figure 17 A-E. Attached to the stylet is a thin oesophageal tube. In all except the Neotylenchoid oesophagus (Fig. 16C), this tube leads to an oval "Valve" in a "median oesophageal bulb", then continues on to the intestine.

Males of some species of the Phasmidia may have a distinct bursa as shown in figure 14 and in figure 19D. Mature females of some genera have enlarged bodies as shown in figure 12.

In the Aphasmidia, the most common types of oesophagi are the "Plectoid", "Cylindrical" and "Dorylaimoid" as shown in figure 17A-C.

Note that the "Plectoid" oesophagus has a basal bulb as in the Rhabditoid type, but that the valve is elongated (Fig. 17C).

The cylindrical type of oesophagus is easy to recognize. It is seen most often in the genus Mononchus, where it is associated with a large mouth cavity containing a tooth as shown in figure 17A.

The "Dorylaimoid" type of oesophagus consists of a narrow anterior portion and a broader posterior portion as shown in figure 17B. This is often, though not always associated with stylets of the types shown in figure 18F-H.

If the nematode belongs to the Phasmidia and has a stylet it will be placed in the Tylenchida, as shown by Item 2 of the Key. If the stylet is absent, it belongs to the Rhabditida.

Nematodes belonging to the Tylenchida are separated into Tylenchoidea and Aphelenchoidea by the characters named in Item 3 of the Key. If the stylet has well developed knobs as shown in figure 18A-D, the nematode belongs to the Tylenchoidea. The oesophageal tube in this group usually has an abrupt bend just posterior to the stylet. At this point can be seen a short branch which is the opening of the dorsal oesophageal gland. If this is present and the stylet knobs are absent, the nematode should have a long tail as shown in figure 19E-F.

On the other hand, if the nematode has a large prominent oesophageal bulb and a stylet with very small knobs or no knobs, it belongs in the Aphelenchoidea (Fig. 16B).

Phasmidia without stylets are placed in the Diplogasteridae if there is no valve in the basal oesophageal bulb (Fig. 15B).

If there is a valve in the dorsal oesophageal bulb, and the stoma is cylindrical as shown in figure 15A, the nematode is placed in the Rhabditoidea. If the stoma is not cylindrical, the nematode is placed in the Cephalobidae.

Nematodes belonging to the Aphasmidia are placed in the plectinae if the oesophagus is as shown in figure 18C. Most nematodes of the group have a spinneret on the tail as shown in figure 19H.

If the oesophagus is cylindrical, as shown in figure 17A, the nematode is placed in the Mononchidae.

If the oesophagus is dorylaimoid, as shown in figure 17B, the nematode is placed in the Dorylaimoidea.

To identify a nematode:

1. Study the oesophagus. Look at four or five specimens if possible.

2. Compare the oesophagus with figures 15, 16 and 17, and decide which type it is.

3. Use the key to identify the nematode to superfamily or family.

4. Find the section relating to this group in "Soil and Freshwater Nematodes".

5. Compare your nematode with the illustrations in the book. This will usually result in an identification to genus.

Key to the Most Common Nematodes of Agricultural Soils & Plants

1. Oesophagus rhabditoid (with valve in basal bulb as in Fig. 15A), diplogasteroid (without valve in basal bulb as in Fig. 15B), tylenchoid (Fig. 16A &B), or aphelenchoid (Fig. 16C). If tylenchoid or aphelenchoid, always with stylet. Males of some genera have a distinct bursa (Fig. 19D). Phasmids always present but most often difficult to locate. Mature females of some genera have a much enlarged body . . . . Phasmidia 2.

Oesophagus plectoid (Fig. 17C), cylindrical (Fig. 17A) or dorylaimoid (Fig. 17B). Often with setae. Males without bursa. Always with elongate, vermiform body . . . . Aphasmidia 6.

2. Stylet always present. Shapes and proportions of stylets vary greatly with genera and species, but are usually recognizable as variations of the forms shown in Fig. 17A-E. Attached to the stylet is a thin oesophageal tube which may be straight or coiled . . . . Tylenchida 3.

Stylet absent. Anterior portion of oesophagus muscular (striated) (Fig. 15A-B) . . . . Rhabditida 4.

3. Stylet mostly with well-developed knobs. If stylet knobs are absent, tail is long and thin (Fig. 19E &F). Dorsal oesophageal gland orifice near base of stylet, or at most, not more than one stylet length posterior to stylet knobs. The oesophageal tube often has an abrupt bend at this point. (Fig. 16A &B) . . . . Tylenchoidea

Stylet knobs small or absent. Median oesophageal bulb occupying nearly full width of body (Fig. 16B). Tail rounded or conical (Fig. 19A-B). Dorsal oesophageal gland orifice in median bulb and difficult to locate. Oesophageal tube without abrupt bends . . . . Aphelenchoidea

4. Oesophagus rhabditoid, with valve in basal bulb (Fig. 15A). . . . . 5.

Oesophagus diplogasteroid, without valve in basal bulb (Fig. 15B).
. . . . Diplogasteridae

5. Stoma cylindrical, usually much longer than wide (Fig. 15A).
. . . . Rhabditidae

Stoma not cylindrical, or if nearly so, about as long as wide. Lips of some genera have distinct projections ranging in shape from rounded to elaborately ornamented. . . . . Cephalobidae

6. Oesophagus plectoid (with basal bulb, Fig. 17C). Tail tip with a small projection (spinneret) Fig. 19H. . . . . Plectinae

Oesophagus cylindroid (Fig. 17A) or dorylaimoid (Fig. 17B). . . . . 7.

7. Oesophagus cylindroid. Mouth cavity large, subglobular, usually with one or more large teeth (Fig. 17A). . . . . Mononchidae

Oesophagus dorylaimoid (Fig. 17B). Stylet often as shown in Fig. 13C. Some with much longer stylet, Fig. 18F-H, others with a tooth. . . Dorylaimoidea

Note: Known plant parasites are either Tylenchida, or species of the genera *Xiphinema*, *Longidorus* and *Trichodorus* of the Dorylaimoidea.

IDENTIFICATION OF PLANT PARASITIC NEMATODES *

Nematodes which are plant parasites always have a stylet which can be recognized as some variation of the stylet shapes shown in Figure 18. Nematodes which do not have stylets, or which have stylets which are not obvious variations of the shapes shown in Figure 18 are not plant parasites. Having determined that the stylet resembles those shown in Figure 18, the nematode can be identified to genus by use of the key.

In using the key, constant reference should be made to the illustrations. When a tentative identification has been made, the nematode should be compared with the illustrations in "Soil and Freshwater Nematodes", by T. Goodey (published in 1951 by Methuen & Co., Ltd., London, and John Wiley & Sons, Inc., New York). This book illustrates only one species of each genus but other species of the genus will be very similar in most respects.

If a stylet is present, it is easily visible on good specimens at magnification of about 400 times. It is always good practice to examine several specimens; characters may be difficult to see on one specimen and easy to see on another. It should also be noted that the key can be used only for the identification of mature females. This is the stage most frequently encountered; for some of the species males are very rare.

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* As used here the term "plant parasites" includes parasites of fungi. It should be kept in mind that many kinds of nematodes probably feed only on fungi, others may feed either on fungi or higher plants. Probably most of the Neotylenchidae feed only on fungi, as do most species of Aphelenchus Certain species of Aphelenchoides and Ditylenchus are known to feed either on fungi or higher plants, and the same may be true for species of other genera of the Tylenchida as well. Thus, nematodes with stylets as shown in Figure 18 may or may not be parasites of higher plants.

Key to the Mature Females of the Common Plant Parasitic Nematodes

1. With median oesophageal bulb (Figs. 16A & C). 5

   Without median oesophageal bulb as in figure 16B; Or without median oesophageal bulb and with stylet as in Fig. 18F, G or H. 2

2. Stylet short (Fig. 16B). . . . . Neotylenchidae

   Stylet long (Fig. 18F-H) 3

3. Stylet straight without enlargement at base (Fig. 18F). .Longidorus

   Stylet with enlargement at base 4

4. Stylet straight with oval enlargements at base (Fig. 18G). Xiphinema

   Stylet curved (Fig. 18H) . . . . Trichodorus

5. Body of mature female pear-shaped, lemon-shaped or enlarged and saccate. (Fig. 12A-G.) Found in roots of plants, either embedded or attached by neck, some as cysts in soil 6

   Body of mature female much longer than wide (Fig. 12 H-L) 10

6. Body of mature female pear-or lemon-shaped (Fig. 12A-C) 7

   Body of mature female saccate (Fig. 12D-G) 8

7. Body of mature female pear-shaped, white, (Fig. 12A). Found completely embedded in roots, nearly always in distinct knots . . . Meloidogyne

   Body of mature female pear-shaped or lemon-shaped, (Fig. 12B-C) white, yellowish, or brown according to age, attached to root by neck only, or found as a brown cyst in soil . . . . Heterodera

8. Mature female embedded in plant root, often in knot, body shape ovoid to spheroid with elongated "tail", vulva nearly terminal. (Fig. 12F-G) . . . . Nacobbus

   Mature females attached to root by neck, body more or less kidney-shaped. (Fig. 12D-E) 9

9. Vulva at 90% of body length, (Fig. 12E) parasites of citrus and olives . . . . Tylenchulus

   Vulva at 72% of body length, parasites of numerous plants, mostly annuals. (Fig. 12D) . . . . Rotylenchulus

10. Tail long and thin, more than 6 times anal body diameter (Fig. 19E,F). . . 11

Tail not long and thin, but rounded, or conical and more or less pointed (Fig. 19, A, B, C, G, H, I) 12

11. Tail tip frequently clavate, (Fig. 19F), one or two ovaries, distance from anterior end to center of median oesophageal bulb equal to, or greater than distance from center of bulb to base of oesophagus . . . . Psilenchus

Tail tip usually pointed, often curved ventrally (Fig. 19E). Distance from anterior end to middle of median oesophageal bulb less than distance from this point to base of oesophagus. One ovary .. .Tylenchus

12. Vulva at 75% or more of body length 13

Vulva at 60% or less of body length 23

13. Body short and stout, length about 6 to 10 times greatest width 14

Body slender, length more than 20 times greatest width (Fig. 12K,L). . .. 16

14. Body with prominent retrorse (directed posteriorly) annules, usually longer than 0.3 mm.; length 10 or more times greatest width. Found in soil . . 15

Body without prominent annules, length 6 to 8 times greatest width. Length about 0.3 mm. Found in roots . . . . Cacopaurus

15. Annules with prominent spines or scales . . . . Criconema

Annules without spines or scales (Fig. 12I) . . . . Criconemoides

16. Mature female more than 2 mm., often 3-5 mm. long. Found in galls, in leaves, or in inflorescense of grains and grasses . . . . Anguina

Body length less than 2 mm. Found in soil or in roots and tubers; sometimes in bulbs, leaves and stems 17

17. Stylet longer than 3 times width of lip region (Fig. 18C) 18

Stylet shorter or about twice width of lip region (Fig. 18A,E) 19

18. Posterior portion of body strongly curved ventrally . . . . . Paratylenchus

Body usually nearly straight, usually covered by loose cuticle of fourth molt . . . . Hemicycliophora

19. Stylet without knobs or with very small knobs; median oesophageal bulb occupying nearly full width of body cavity (Fig. 16C, 18E). 20

Stylet with distinct knobs; median oesophageal bulb occupying less than 2/3 of width of body cavity (Fig. 16A) 21

20. Tail pointed (Fig. 19G) . . . . Aphelenchoides

Tail rounded (Fig. 19B) . . . . Aphelenchus

21. Tail conical, pointed (Fig. 19G) body long and slender; length 40 or more times greatest width. Endoparasitic in bulbs, stems, leaves and tubers, from mushroom compost, or sometimes in soil . . . . Ditylenchus

Tail tip rounded 22

22. Lip region distinctly set off from body; oesophageal glands forming a lobe overlapping intestine; knobs of stylet closely joined. A common genus endoparasitic in roots and tubers, also found in soil (Fig. 18A) . . . . Pratylenchus

Lip region not distinctly set off from body; oesophageal gland forming a distinct basal bulb (Fig. 16A) knobs of stylet separated like an inverted Y. A very rare genus from soil . . . . Chitinotylenchus

23. At least 2 mm. long, slim; length 45 or more times greatest width. Stylet very long, six or more times as long as width of lip region (Fig. 18D). . 24

Less than 1.5 mm. long, length of stylet not more than 5 times width of lip region 25

24. Tail pointed, (Fig. 18C) oesophagus with distinct basal bulb. . Dolichodorus

Tail rounded, base of oesophagus a lobe overlapping intestine . . . . Belonolaimus

25. Lip region flattened, stylet length about twice width of lip region . .
. . . . *Radopholus*

Lip region convex conoid, stylet length 3 or more times width of lip
region 26

26. Tail 2 or more times as long as anal body diameter, tapering (Fig. 19G). . 27

Tail shorter than anal body diameter, rounded (Fig. 19A) 28

27. Tail tip rounded . . . . *Tylenchorhynchus*

Tail tip nearly pointed . . . . *Tetylenchus*

28. Lip region distinctly set off from body, divided into minute plates.
Body at rest or fixed lying only slightly curved . . . . . . *Hoplolaimus*

Annulated lip region continuous with body contour, body usually lies
in loose spiral when fixed or at rest. (Fig. 12H) 29

29. Opening of dorsal oesophageal gland about one-third stylet length or more
posterior to stylet knobs . . . . *Helicotylenchus*

Opening of dorsal oesophageal gland much less than one-third stylet length
posterior to stylet knobs . . . . *Rotylenchus*

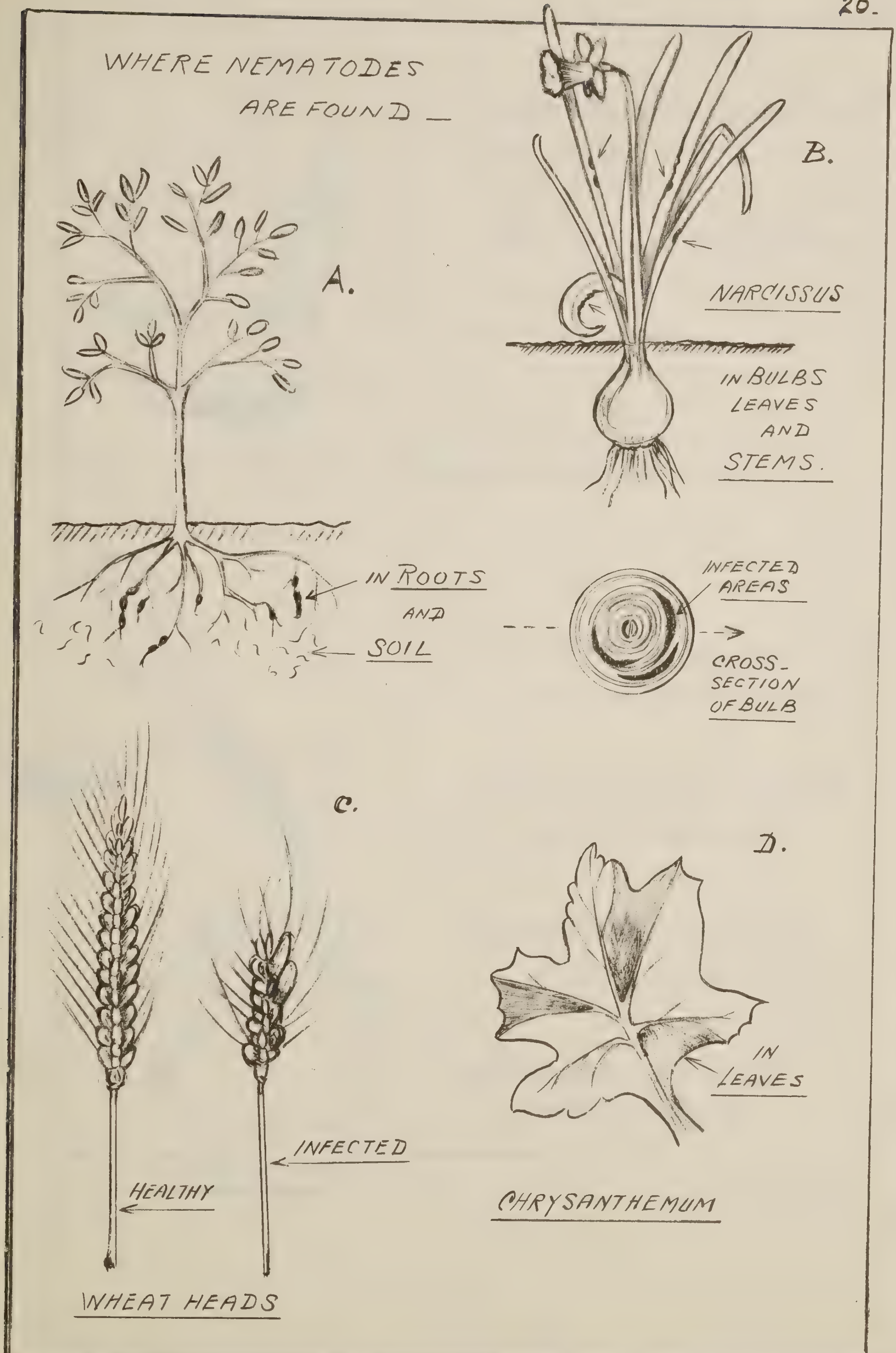
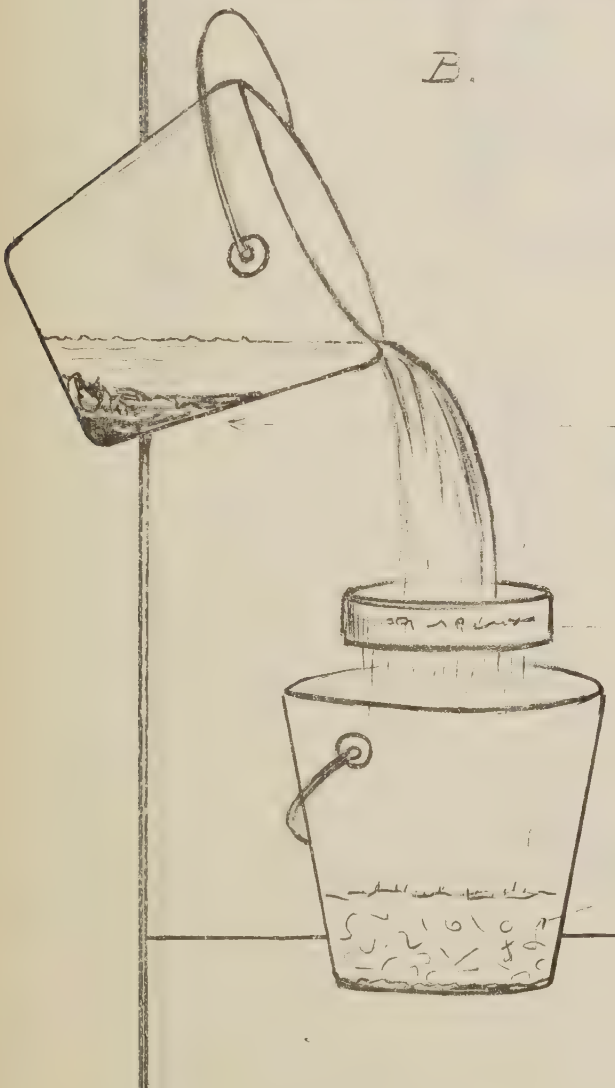

WHERE NEMATODES
ARE FOUND _
A.
IN ROOTS
AND
SOIL
B.
NARCISSUS
IN BULBS
LEAVES
AND
STEMS.
INFECTED
AREAS
CROSS-
SECTION
OF BULB
C.
INFECTED
HEALTHY
WHEAT HEADS
D.
IN
LEAVES
CHRYSANTHEMUM

FIGURE - 1.

A.

PLACE SOIL SAMPLE IN BUCKET,
ADD WATER AND STIR
WITH STICK.
SAND SETTLES TO BOTTOM
NEMATODES REMAIN
SUSPENDED

B.



POUR THROUGH 20-MESH SIEVE
INTO SECOND BUCKET.

SAND AND GRAVEL REMAIN
IN FIRST BUCKET

SIEVE REMOVES LARGE
PIECES OF DEBRIS

NEMATODES PASS
THROUGH SIEVE

FIGURE – 2.

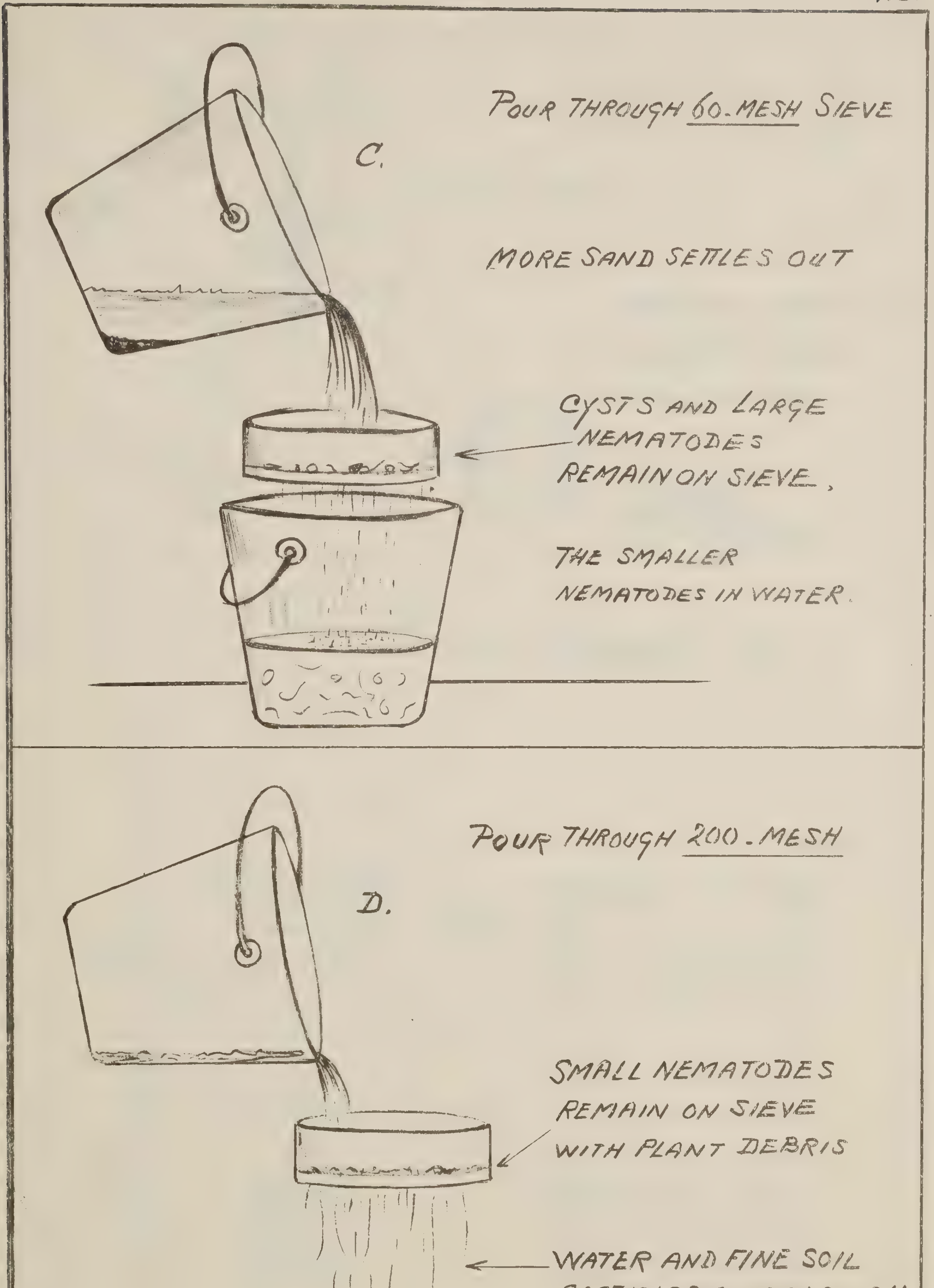

POUR THROUGH 60-MESH SIEVE
C.
MORE SAND SETTLES OUT
CYSTS AND LARGE NEMATODES REMAIN ON SIEVE.
THE SMALLER NEMATODES IN WATER.
POUR THROUGH 200-MESH
D.
SMALL NEMATODES REMAIN ON SIEVE WITH PLANT DEBRIS
WATER AND FINE SOIL PARTICLES PASS THROUGH.

FIGURE - 3.

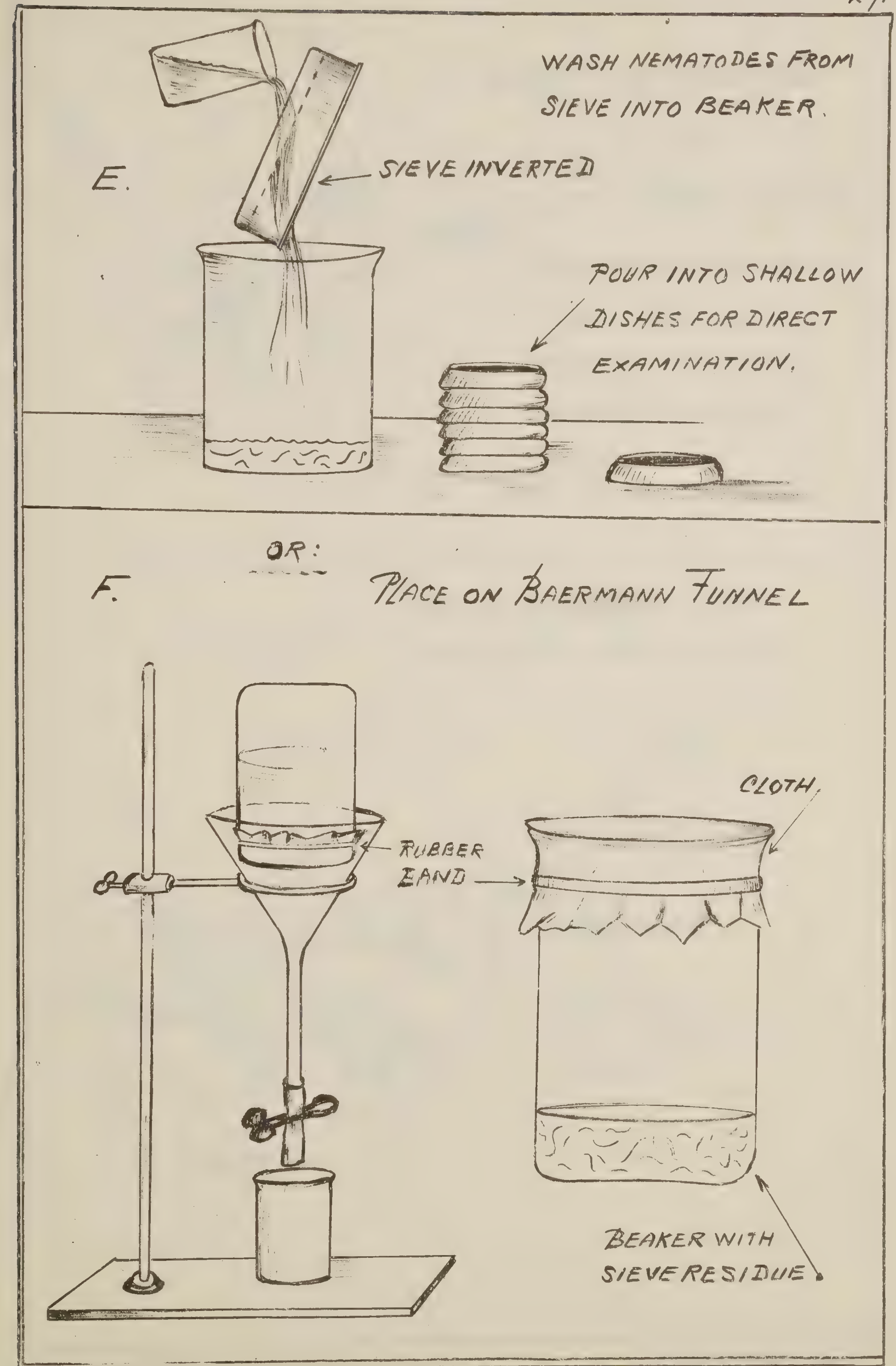

WASH NEMATODES FROM SIEVE INTO BEAKER.
E.
SIEVE INVERTED
POUR INTO SHALLOW DISHES FOR DIRECT EXAMINATION.
OR:
F.
PLACE ON BAERMANN FUNNEL
CLOTH.
RUBBER BAND
BEAKER WITH SIEVE RESIDUE.

FIGURE - 4.

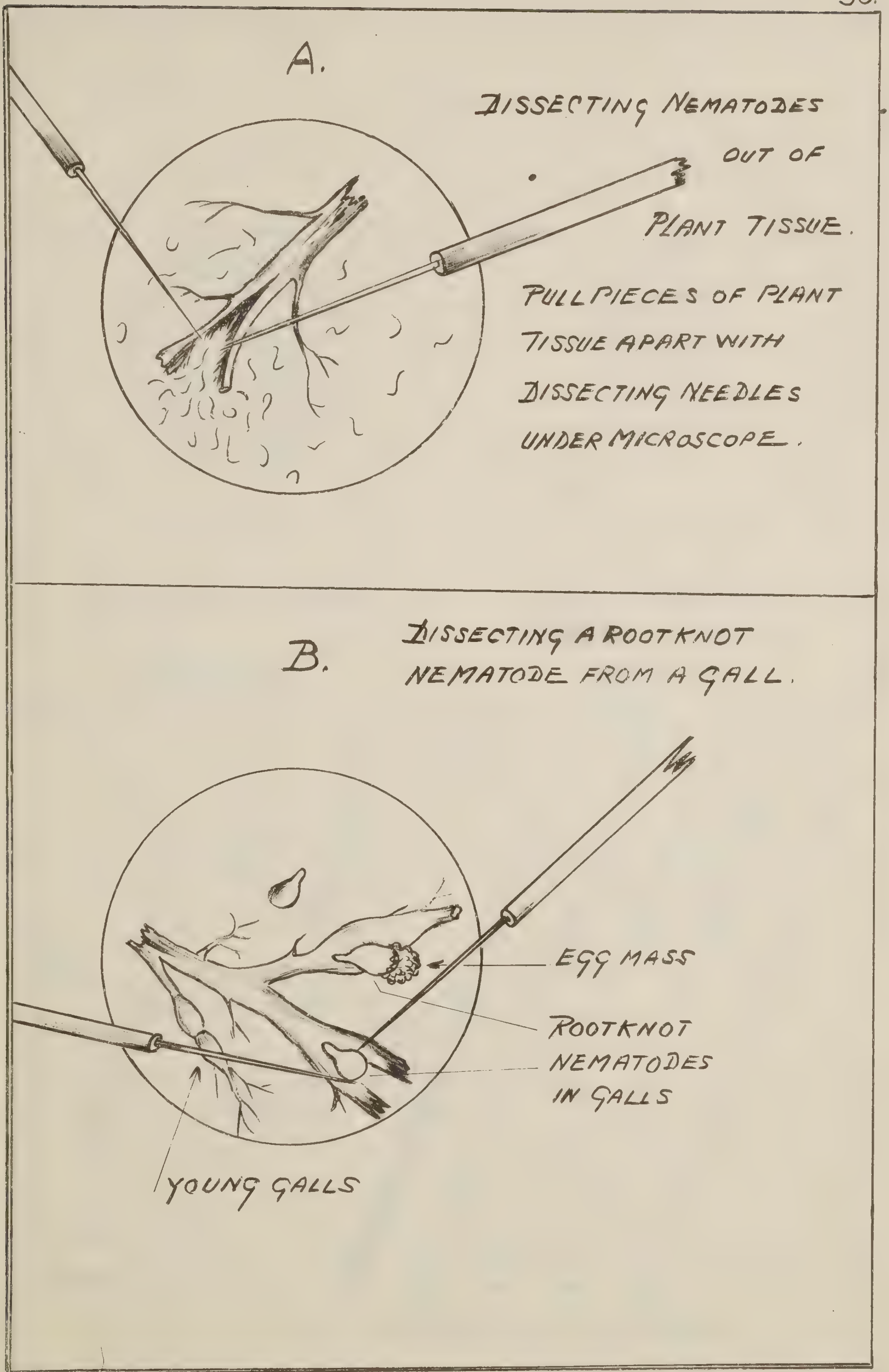

A.
DISSECTING NEMATODES OUT OF PLANT TISSUE.
PULL PIECES OF PLANT TISSUE APART WITH DISSECTING NEEDLES UNDER MICROSCOPE.
B.
DISSECTING A ROOTKNOT NEMATODE FROM A GALL.
EGG MASS
ROOTKNOT NEMATODES IN GALLS
YOUNG GALLS

FIGURE - 5

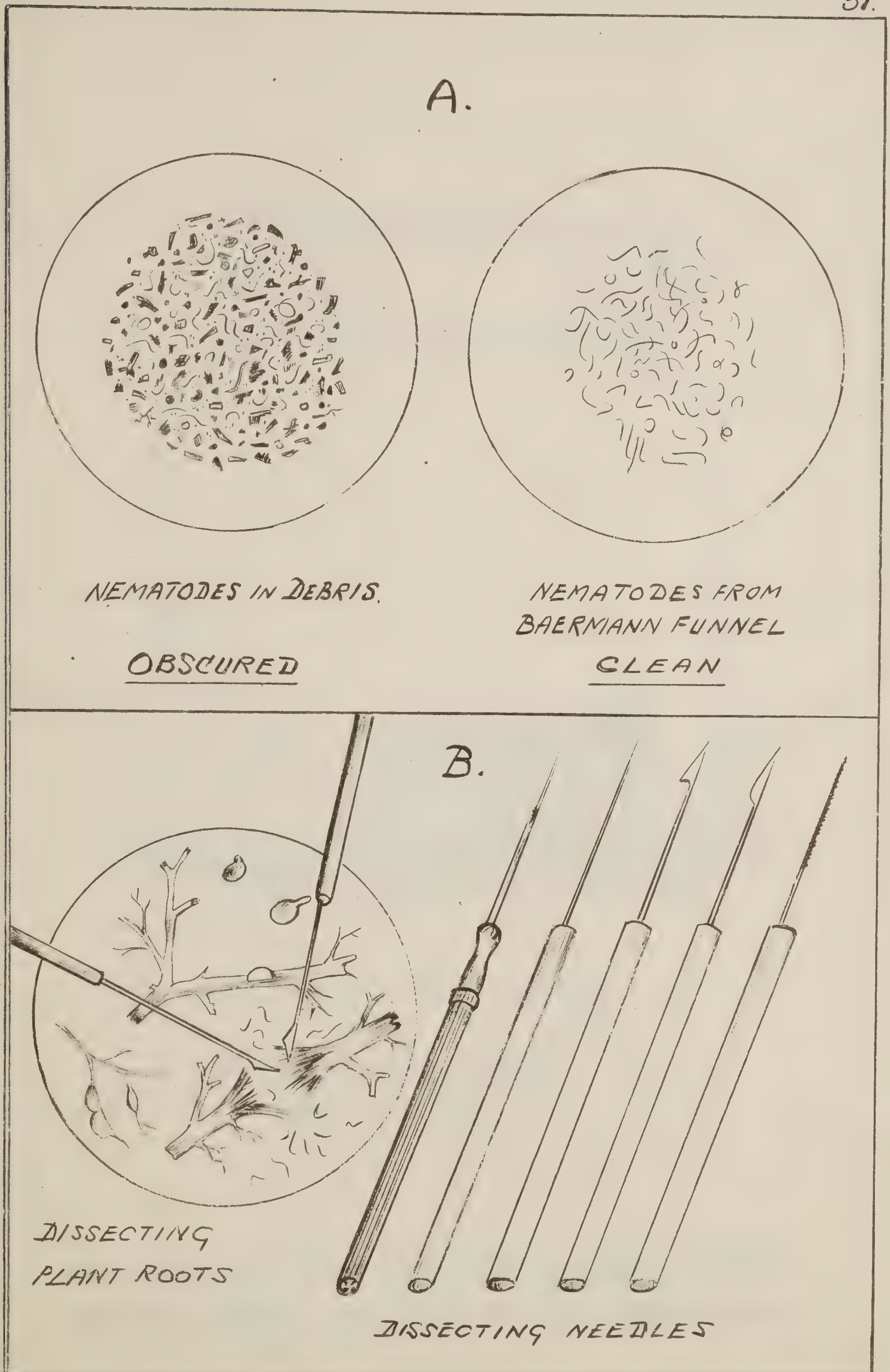
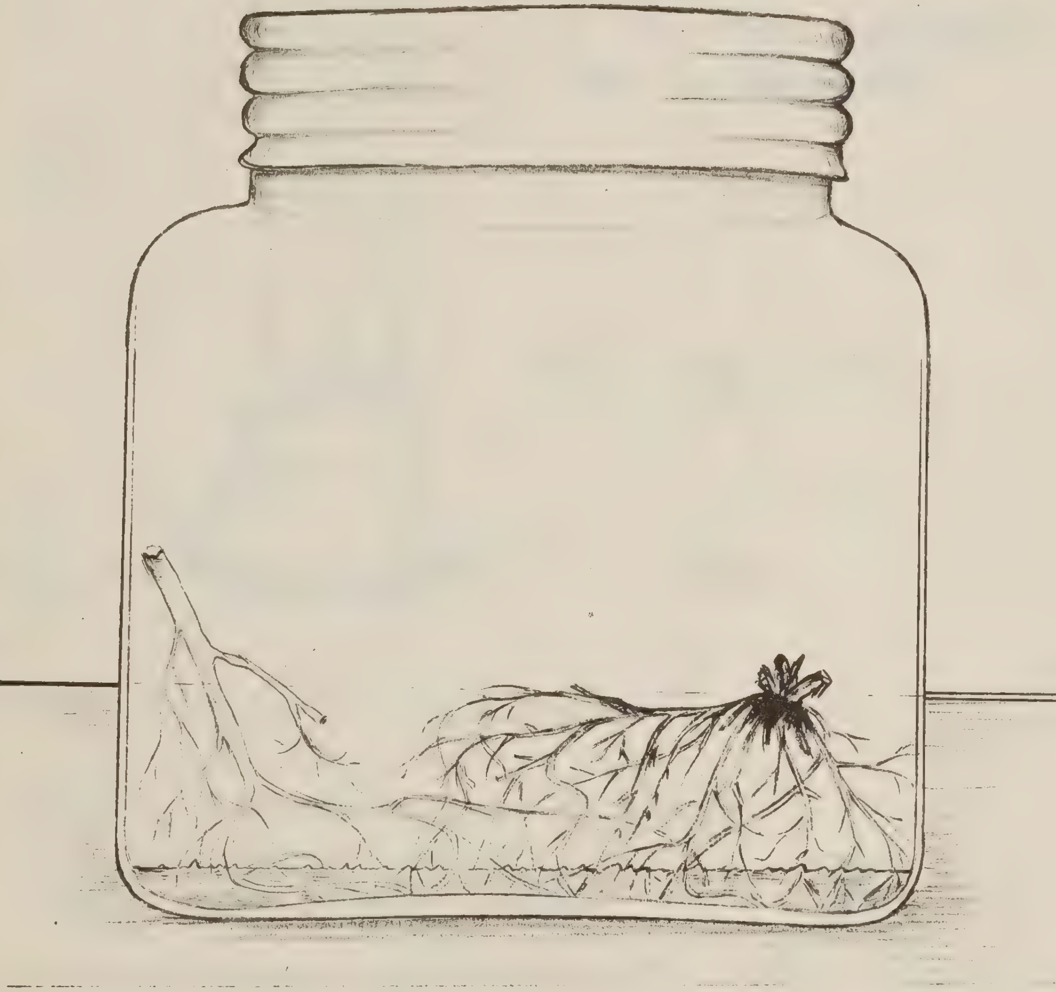

A.
NEMATODES IN DEBRIS.
OBSCURED
NEMATODES FROM
BAERMANN FUNNEL
CLEAN
B.
DISSECTING
PLANT ROOTS
DISSECTING NEEDLES

FIGURE - 6.



INCUBATION TECHNIQUE
FOR
EXTRACTING NEMATODES
FROM ROOTS

WET ROOTS IN FRUIT JAR; ADD A LITTLE WATER;
POUR OFF WATER AND EXAMINE FOR NEMATODES.

FIGURE -7.

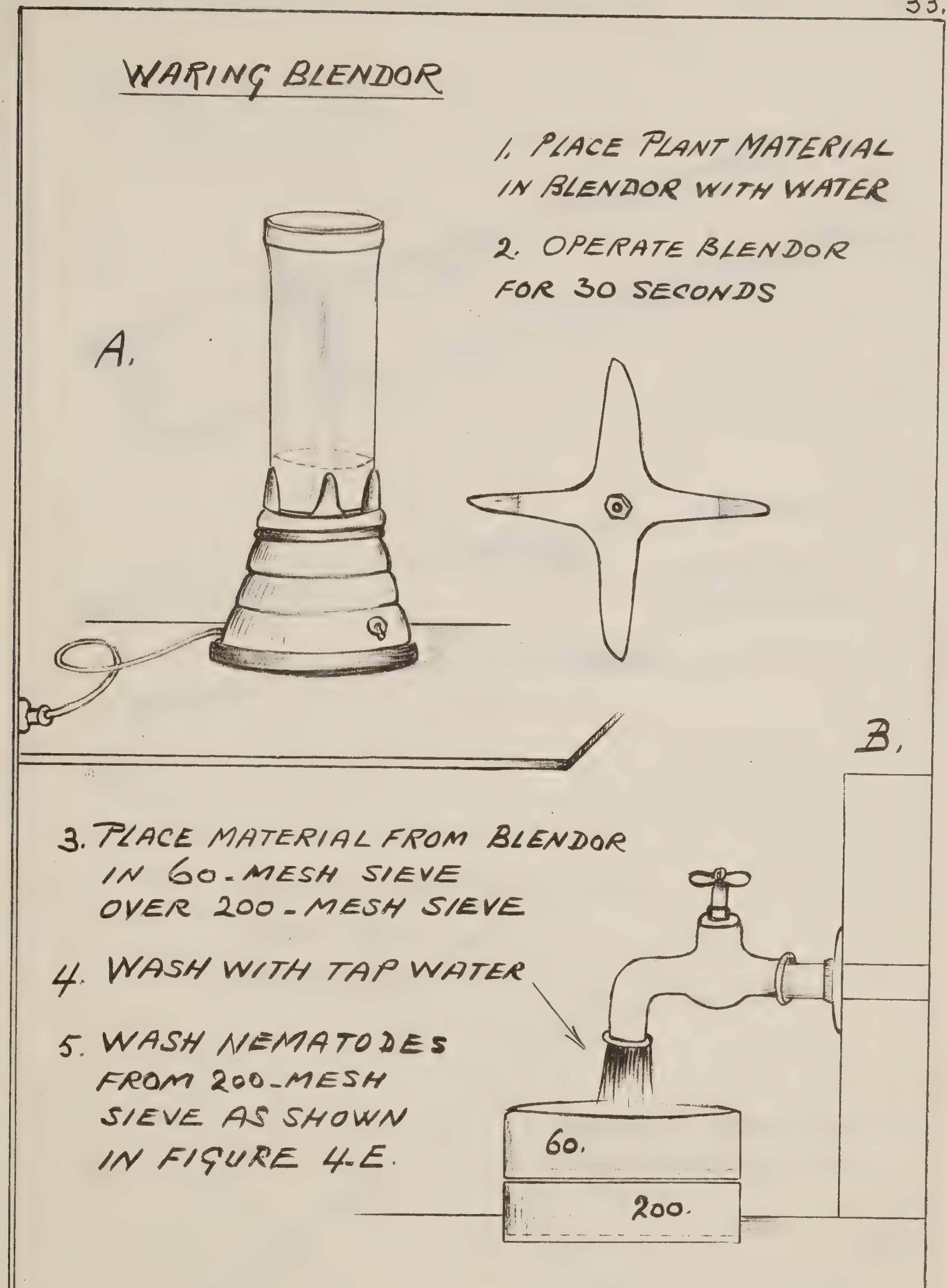

WARING BLENDOR
1. PLACE PLANT MATERIAL IN BLENDOR WITH WATER
2. OPERATE BLENDOR FOR 30 SECONDS
A.
B.
3. PLACE MATERIAL FROM BLENDOR IN 60-MESH SIEVE OVER 200-MESH SIEVE
4. WASH WITH TAP WATER
5. WASH NEMATODES FROM 200-MESH SIEVE AS SHOWN IN FIGURE 4-E.
60.
200.

FIGURE - 8.

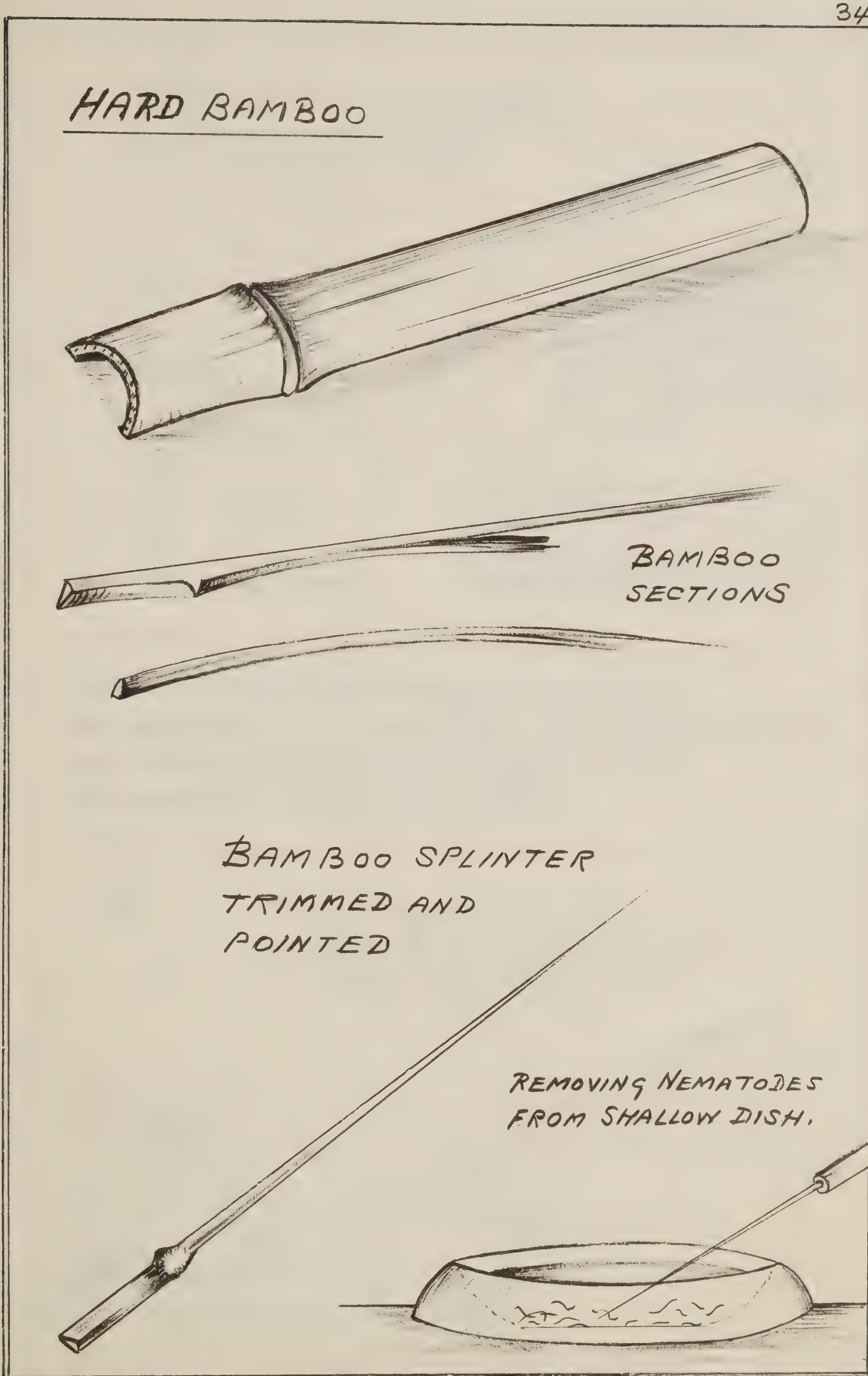
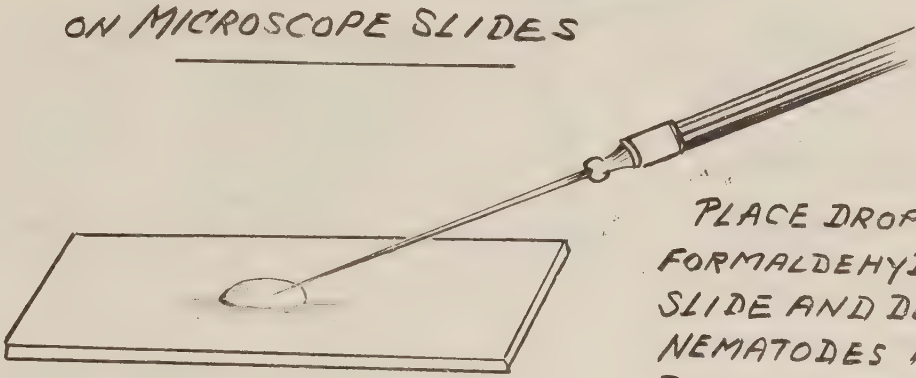

HARD BAMBOO
BAMBOO SECTIONS
BAMBOO SPLINTER TRIMMED AND POINTED
REMOVING NEMATODES FROM SHALLOW DISH.

FIGURE - 9.

MOUNTING NEMATODES ON MICROSCOPE SLIDES

A.



PLACE DROP OF 5% FORMALDEHYDE ON SLIDE AND DEPOSIT NEMATODES IN THE DROP, USING BAMBOO SPLINTER.

YOU CAN MOUNT AS MANY AS 20 NEMATODES ON ONE SLIDE.

PIECES OF GLASS ROD TO SUPPORT COVER GLASS (IF DESIRED)

LOOK AT THE DROP UNDER THE DISSECTING MICROSCOPE AND BE SURE THAT THE NEMATODES LIE IN THE BOTTOM OF THE DROP AND IN CONTACT WITH THE SLIDE.

B.

USING FORCEPS, PLACE A COVER GLASS OVER THE DROP.

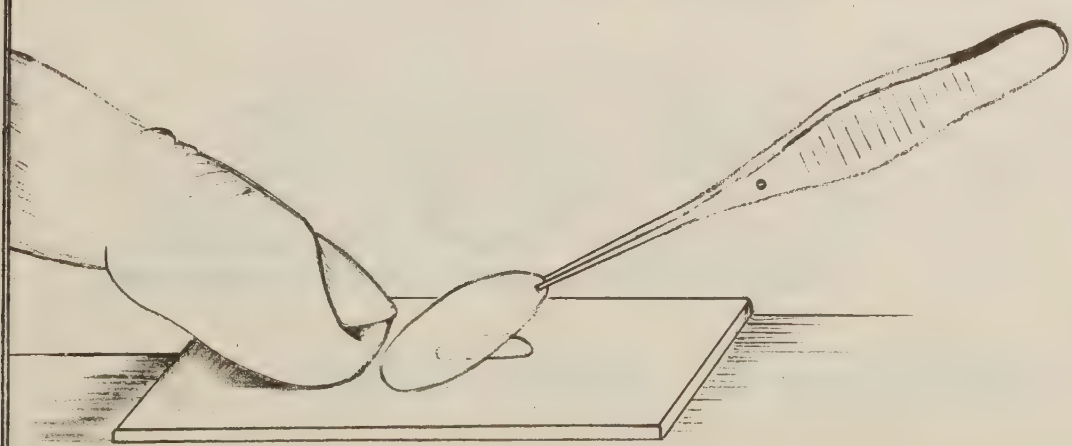
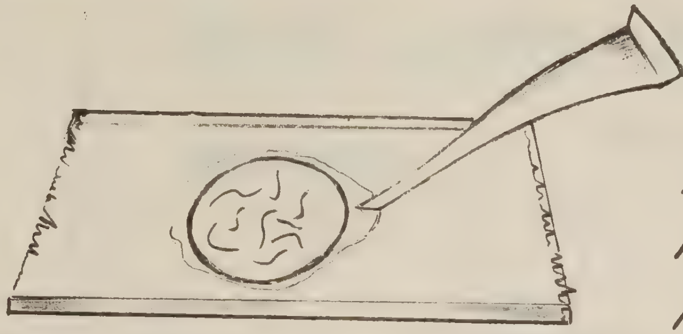


FIGURE_10.

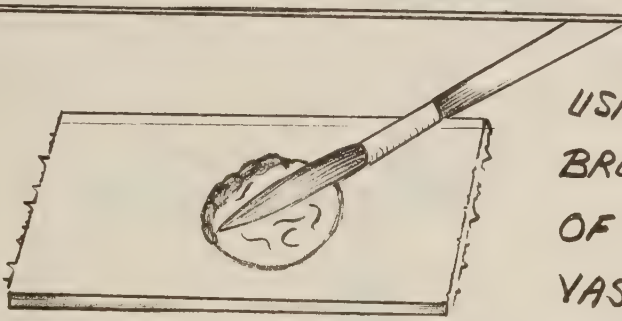


USE A SMALL PIECE OF FILTER PAPER, BLOTTING PAPER OR PAPER TOWEL TO ABSORB EXCESS WATER

DO THIS UNDER THE DISSECTING MICROSCOPE TO BE SURE THAT NO NEMATODES ARE LOST.

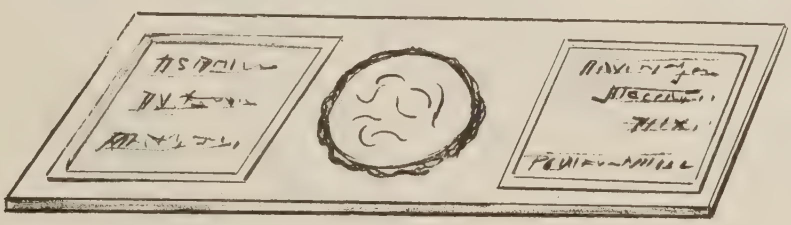
A.

B.



USING A SMALL BRUSH APPLY A RING OF HOT PARAFFIN-VASELINE MIXTURE, ZUT, OR OTHER SEALING MATERIAL.

C.



LABEL SLIDE, USING FINE PEN.

FIGURE 11.

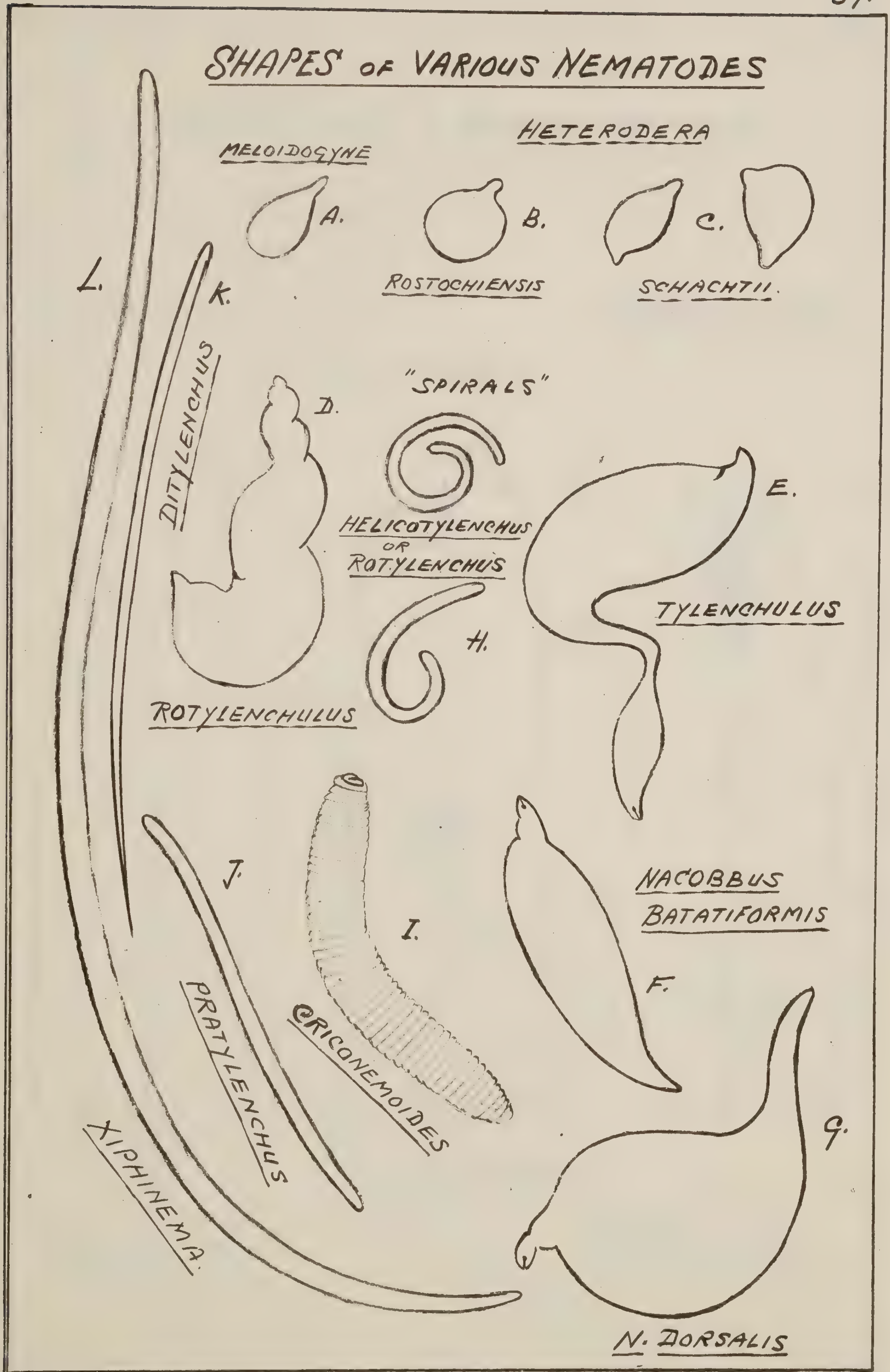

SHAPES OF VARIOUS NEMATODES
MELOIDOGYNE
A.
HETERODERA
B.
ROSTOCHIENSIS
C.
SCHACHTII.
L.
K.
DITYLENCHUS
D.
ROTYLENCHULUS
"SPIRALS"
HELICOTYLENCHUS OR ROTYLENCHUS
H.
E.
TYLENCHULUS
J.
PRATYLENCHUS
I.
CRICONEMOIDES
XIPHINEMA.
NACOBBUS BATATIFORMIS
F.
G.
N. DORSALIS

FIGURE 12.

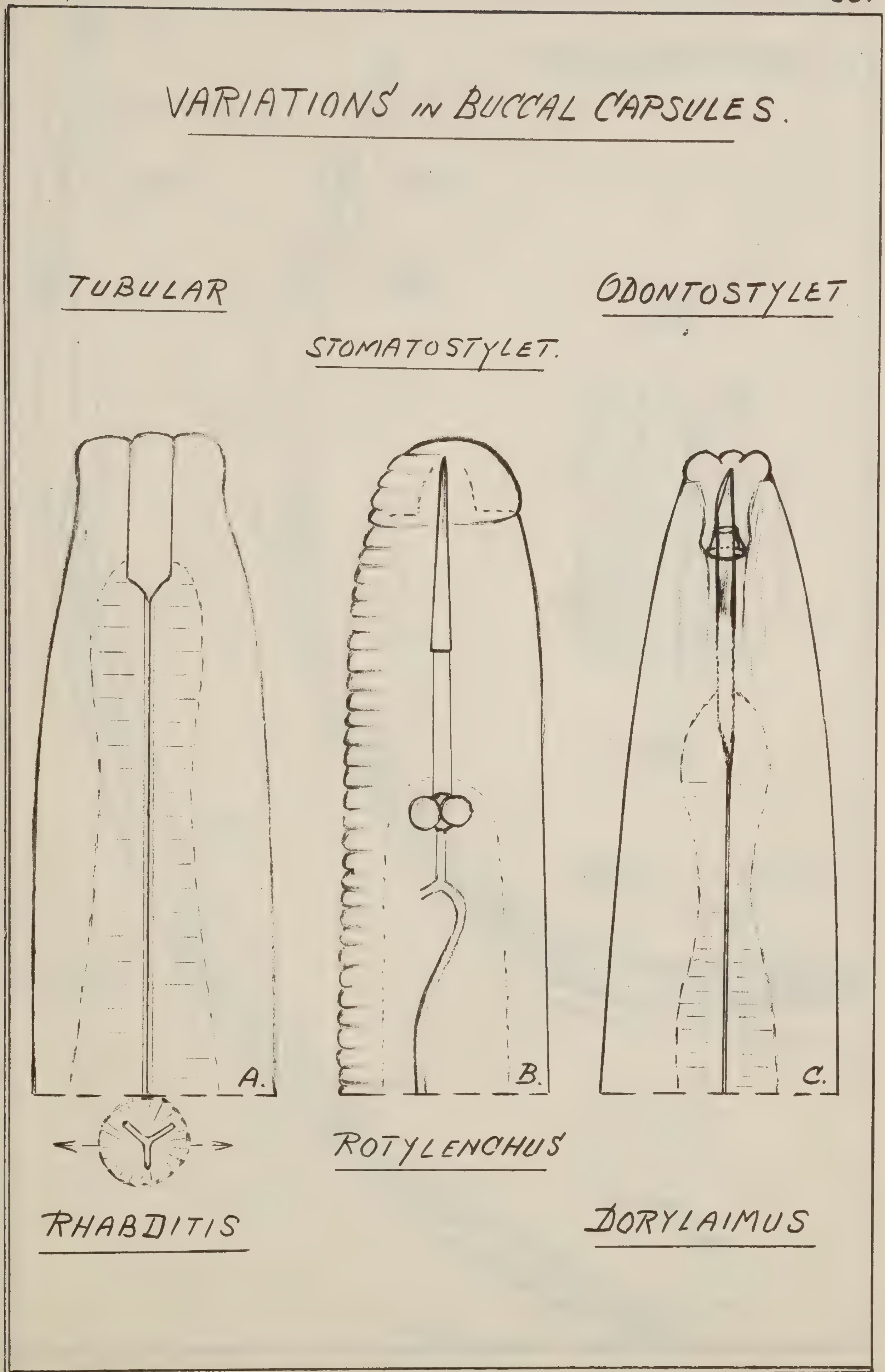

VARIATIONS IN BUCCAL CAPSULES.
TUBULAR
STOMATOSTYLET.
ODONTOSTYLET
A.
B.
C.
ROTYLENCHUS
RHABDITIS
DORYLAIMUS

FIGURE 13.

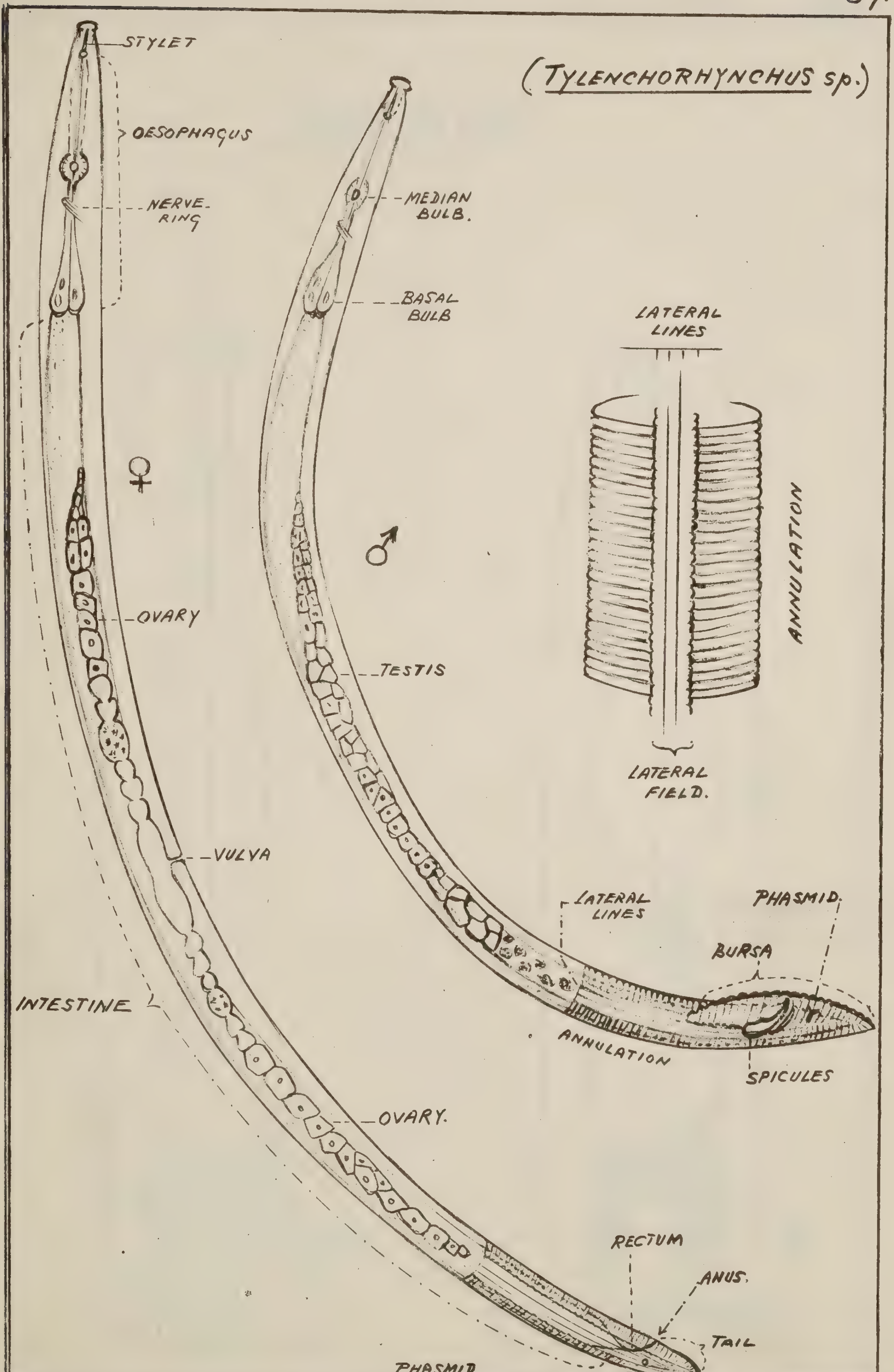
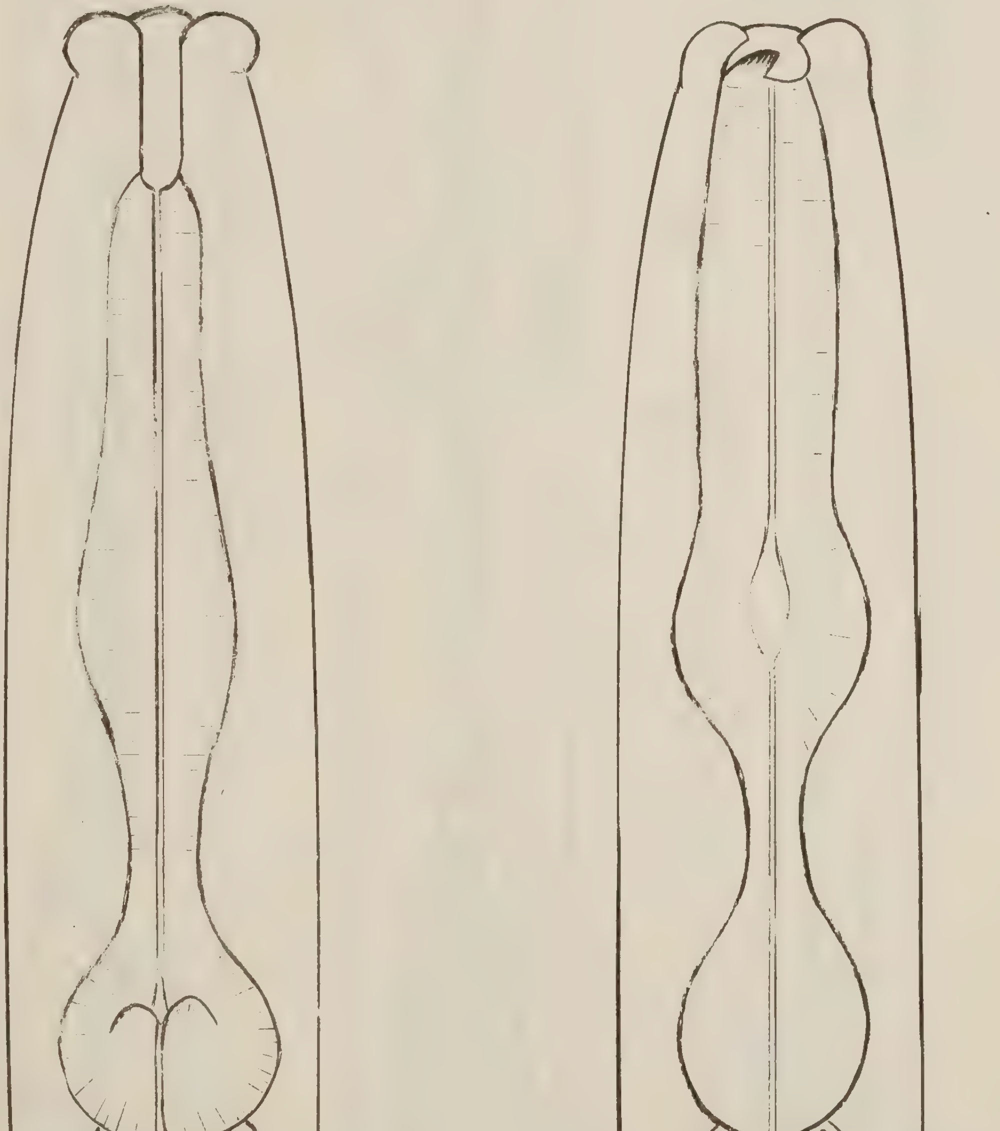

(TYLENCHORHYNCHUS sp.)
STYLET
OESOPHAGUS
NERVE RING
MEDIAN BULB.
BASAL BULB
LATERAL LINES
ANNULATION
LATERAL FIELD.
♀
♂
OVARY
TESTIS
VULVA
LATERAL LINES
PHASMID
BURSA
INTESTINE
ANNULATION
SPICULES
OVARY.
RECTUM
ANUS.
TAIL
PHASMID

FIGURE 14.

PHASMIDIA.



RHABDITOID DIPLOGASTEROID.

FIGURE 15.

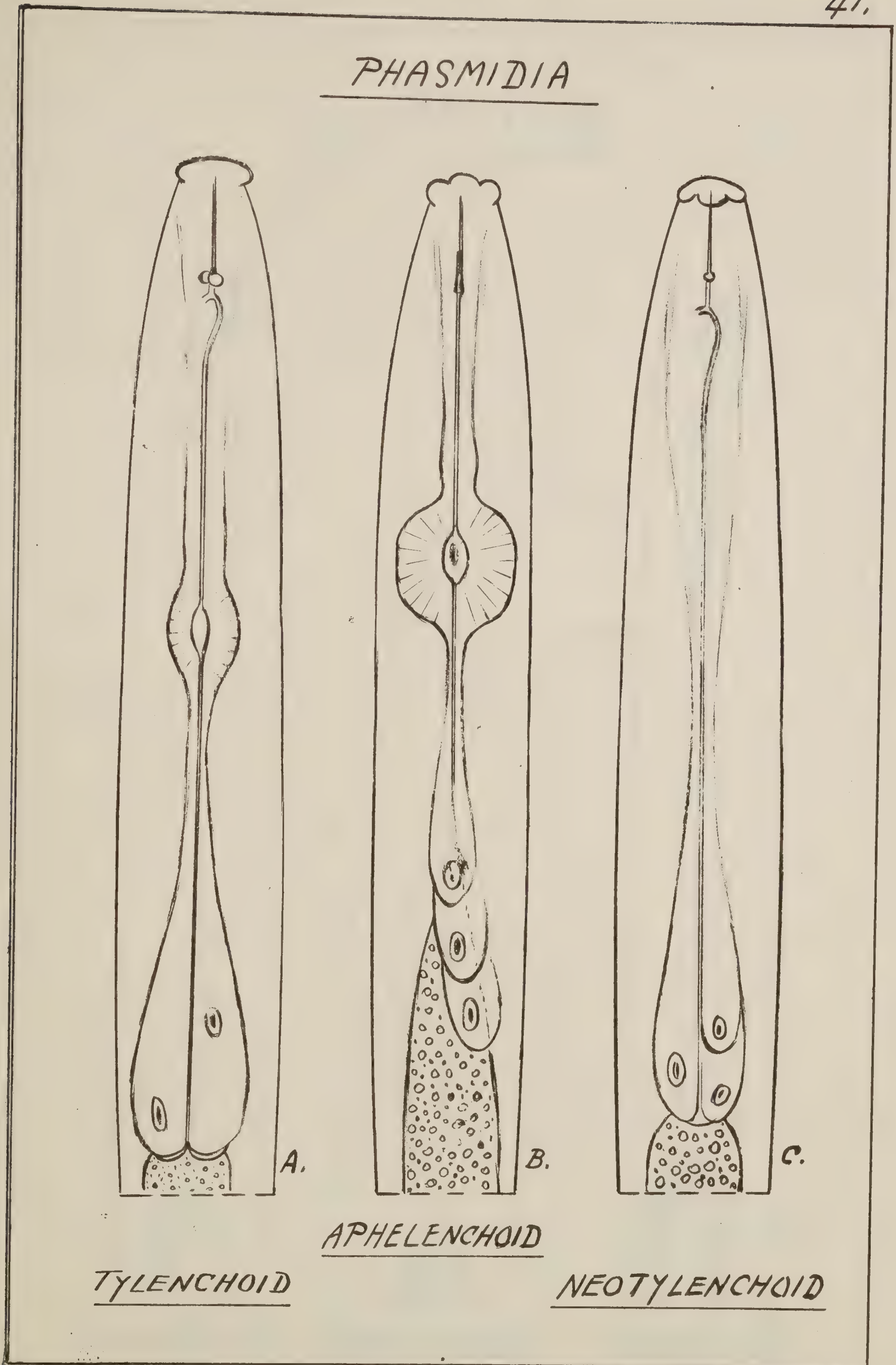
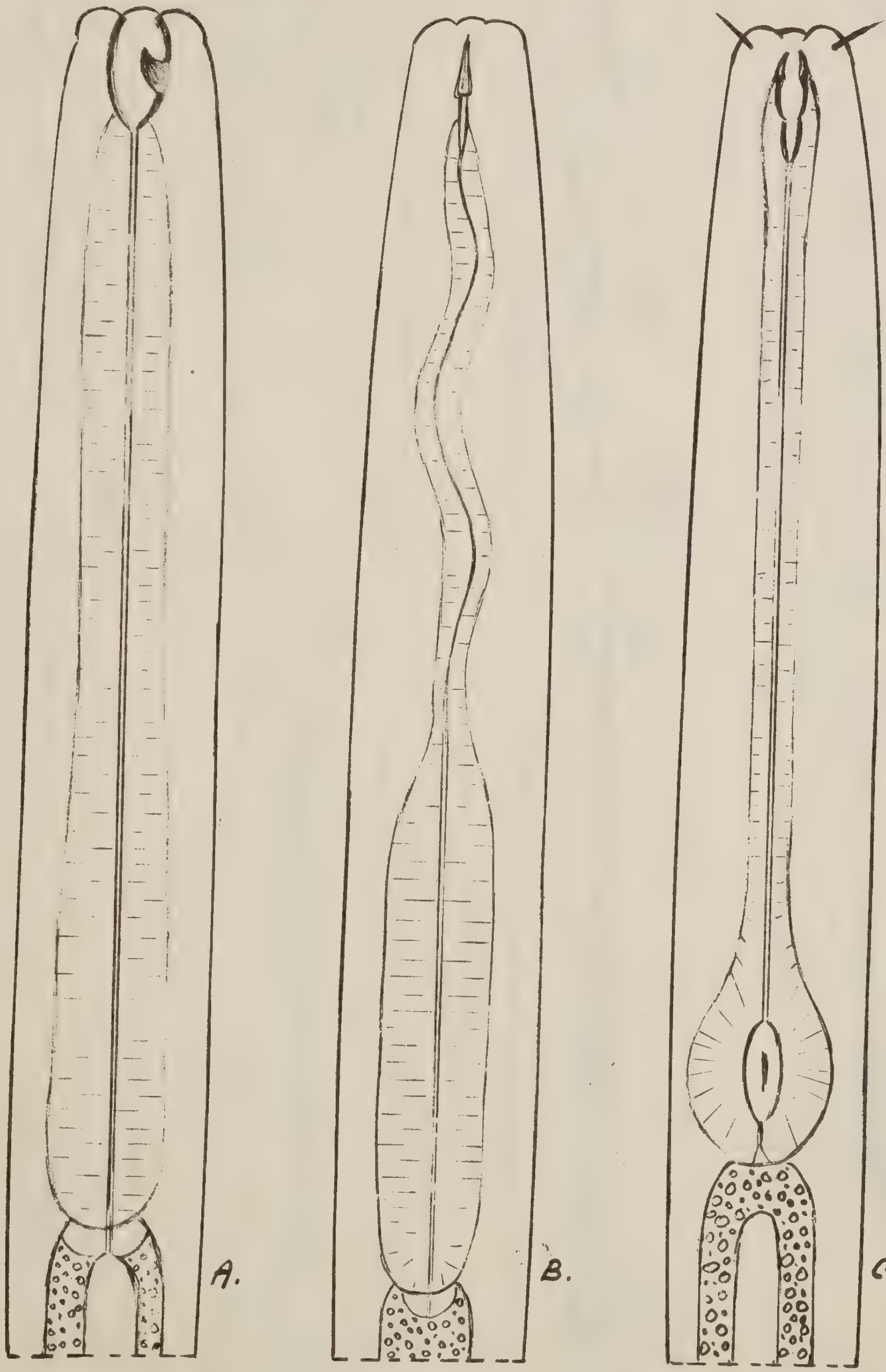


FIGURE 16.

APHASMIDIA.



CYLINDROID. DORYLAIMOID. PLECTOID.

FIGURE 17.

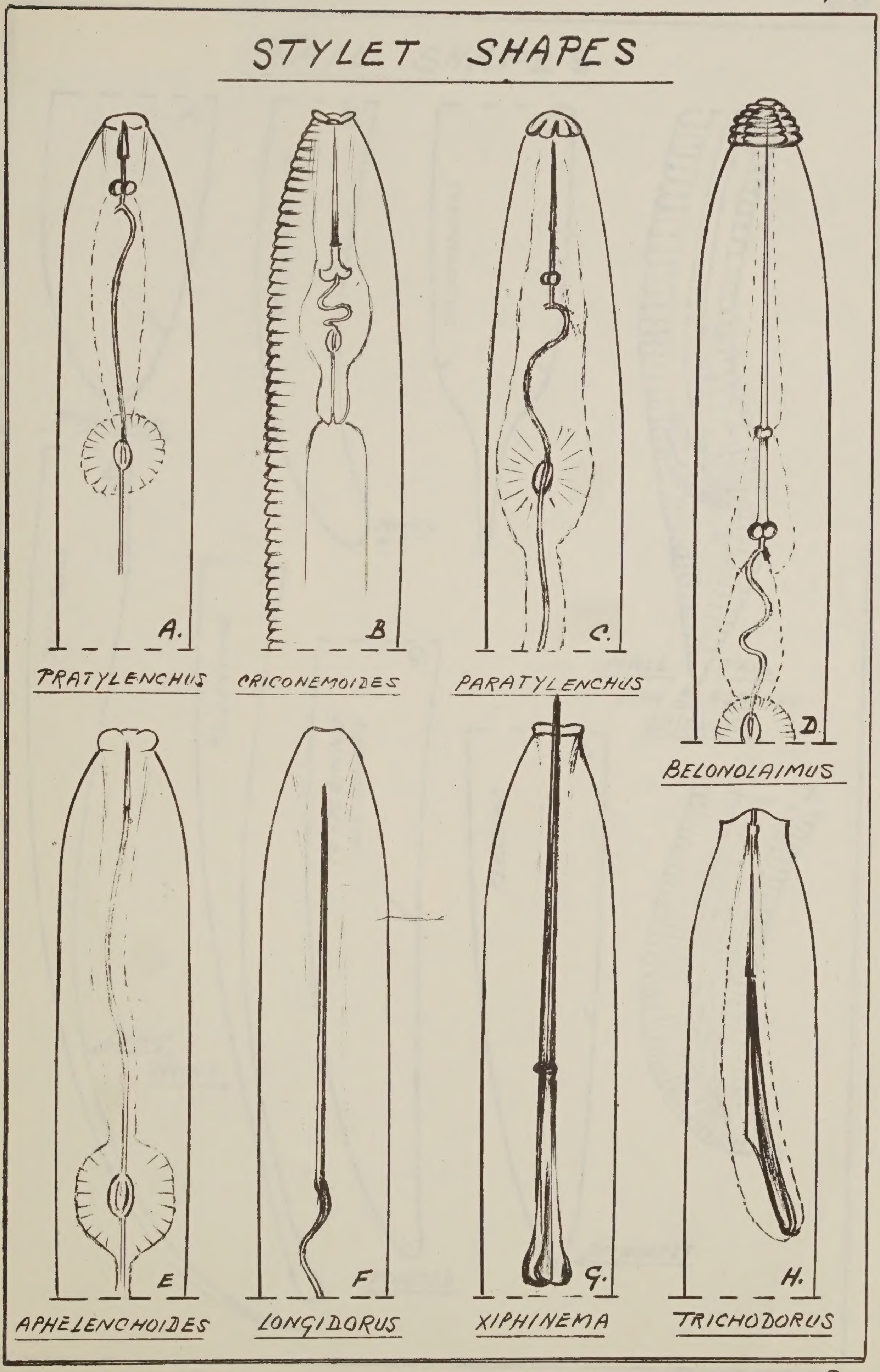
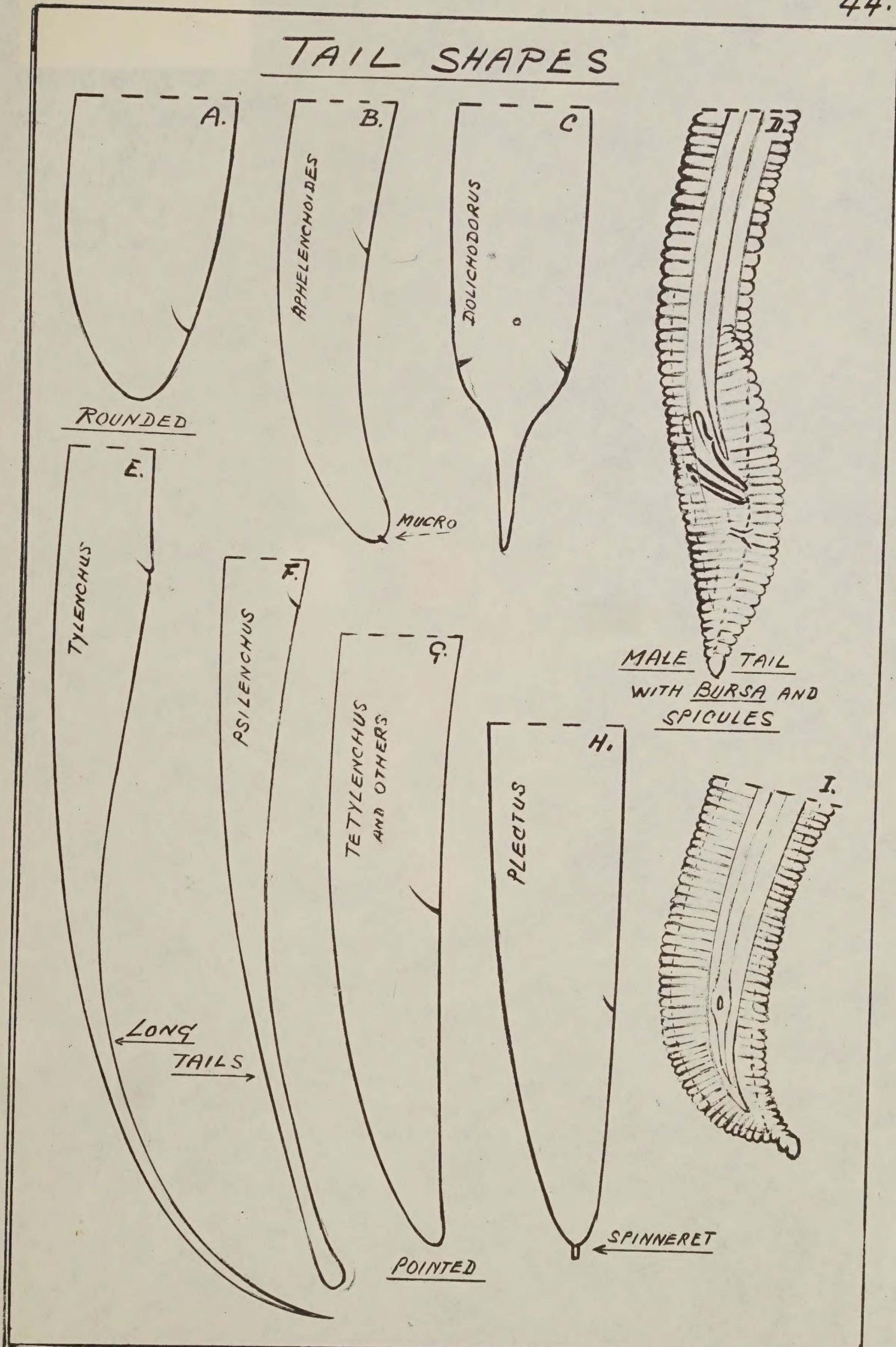

STYLET SHAPES
A.
PRATYLENCHUS
B
CRICONEMOIDES
C.
PARATYLENCHUS
D.
BELONOLAIMUS
E
APHELENCHOIDES
F
LONGIDORUS
G.
XIPHINEMA
H.
TRICHODORUS

FIGURE 18.



TAIL SHAPES
A.
ROUNDED
B.
APHELENCHOIDES
MUCRO
C
DOLICHODORUS
D.
MALE TAIL
WITH BURSA AND SPICULES
E.
TYLENCHUS
F.
PSILENCHUS
G.
TETYLENCHUS AND OTHERS
LONG
TAILS
POINTED
H.
PLECTUS
SPINNERET
I.

FIGURE 19.